TSE examination of small ruminants in Denmark 2001-2018

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TSE examination of small ruminants in Denmark 2001-2018

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During 2001 to 2018 app. 77.000 small ruminants were tested for TSE. All together 13 TSE cases were confirmed (12 fallen stock sheep and 1 slaughter sheep)! All 13 cases were diagnosed atypical scrapie. The most common differential diagnosis among the 50 clinical scrapie suspicions was listeriosis - found in 28% of the cases. Listeriosis was characterized by multifocal, necrotizing, non-suppurative encephalitis confined to the brainstem region.

Classic scrapie has so far never been reported from Denmark.

The aim of this report is to summarize information on the TSE testing of small ruminants performed in Denmark 2001 to 2018. DTU-VET is the national reference laboratory of TSE/Scrapie in Denmark. The Danish TSE surveillance program is carried out in accordance with the demands given by the EU Commission as well as OIE. Over the years the TSE surveillance program has changed and included during 2018 the testing of the following small ruminant categories:

- Small ruminants with a clinical suspicion of TSE without age limit. All cases examined at DTU-VET
- A random sample of fallen stock animals older than 18 months to fulfil the requirements of the TSE legislation, which with the Danish sheep and goat population in 2017 are annually 500 sheep tests and 100 goat tests. The tests are performed at a private, approved laboratory.

The number of animals tested within the categories clinical suspicions, fallen stock and healthy slaughter animals are listed in Table 1 together with the number of TSE positive cases. Table 2 summarizes the data concerning the PrP positive cases.

Did you know that: The annual average Danish sheep population 2001 to 2018 was 150.000 animals. The number of sheep herds in 2018 is ca. 2500 and 84% of the herds have less than 100 sheep. The Danish goat population is ca. 12.000 animals.

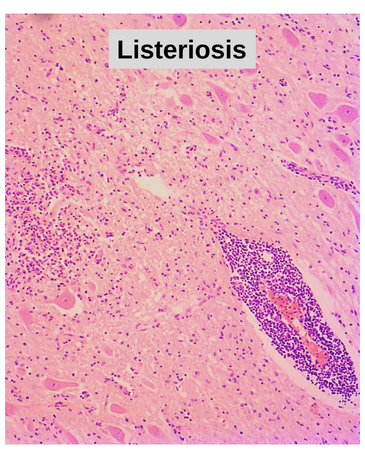
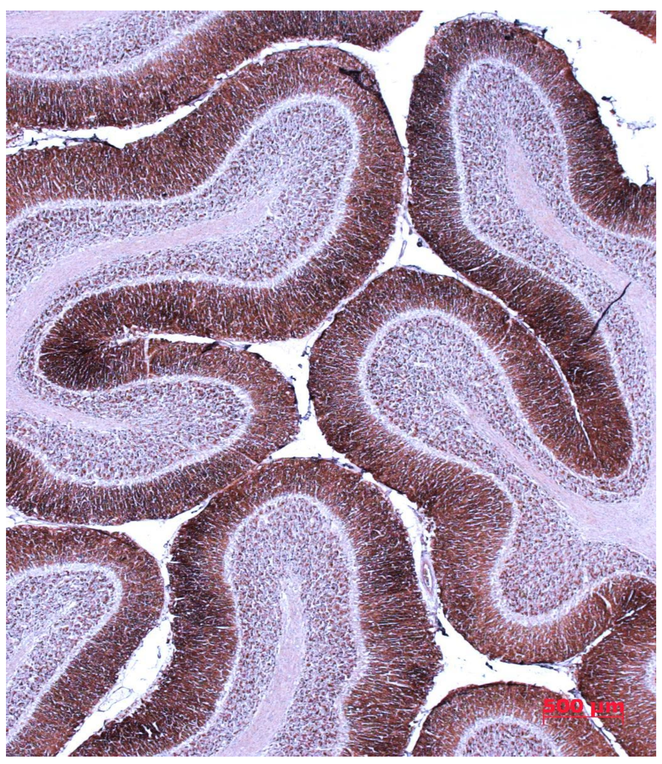
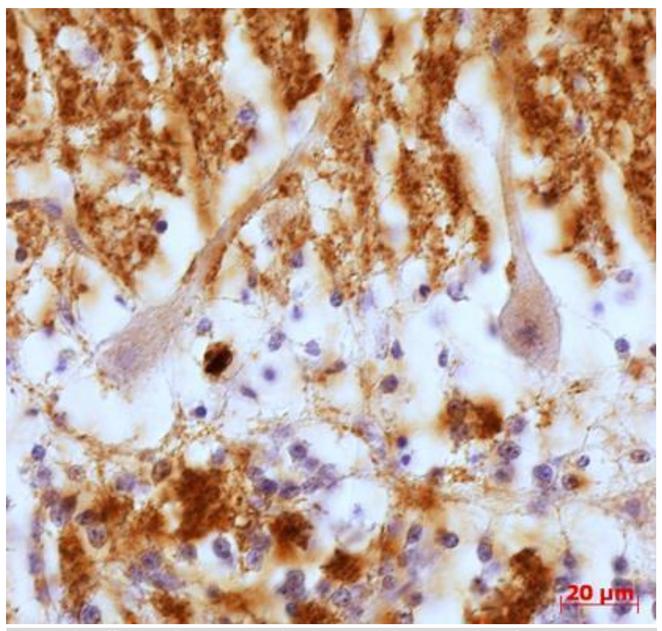


Table 1. Number of small ruminants tested for TSE annually within different categories

Category	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Sum
TSE positive	0	0	0	0	0	3	1	1	0	2	5	0	0	0	0	1	0	0	13
Clinical suspicions	8	11	8	5	2	4	1	0	3	3	1	0	0	2	1	0	1	0	50
Fallen stock	0	492	3000	7500	8000	8000	8000	8000	8000	8000	8000	3800	637	703	771	812	609	300	75.000
Healthy animals	82	641	664	98	310	436	13	0	0	0	0	0	0	0	0	0	0	0	2500





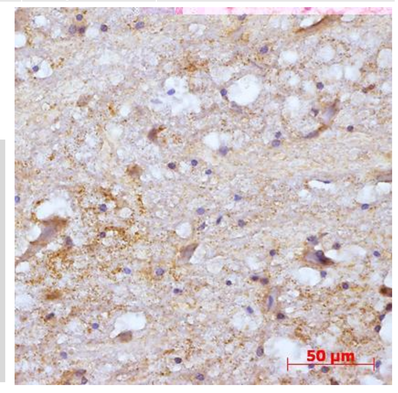
Cerebellum from a sheep with atypical scrapie. Detection of PrP by immunohistochemistry. An intensive accumulation of PrP (brown) is seen in the molecular and granular layers as well as affecting Purkinje cells (bottom).

Table 2. Summary of the 13 indigenous TSE cases in small ruminants 2001 - 2018.

No	Year	Category	Breed	Age (years)	Rapid test private lab.	Histopathology	Additional results regarding TSE	TSE genotype	Conclusion
1	2006	Fallen stock	sheep	?	IDEXX Herdcheck, positive	Not done due to autolysis.	PrP Prionics WB positive, BioRad WB* positive	AHQ/ARQ	Atypical scrapie, no signs of BSE
2	2006	Fallen stock		10	IDEXX Herdcheck, positive	Not done due to autolysis.	PrP Prionics WB negative, OIE-SAF WB positive*, BioRad WB* positive	AHQ/ARQ	Atypical scrapie, no signs of BSE
3	2006	Slaughter animal	sheep	7	IDEXX Herdcheck, positive	PrP staining Cerebellum <<< obex,	IDEXX Herdcheck positive, Prionics WB negative**	AHQ/ARQ	Atypical scrapie, no signs of BSE
4	2007	Fallen stock	sheep	13	IDEXX Herdcheck, positive	Not done due to autolysis.	PrP positiv by IDEXX Herdcheck, negative by Prionics WB. Atypical scrapie by AFSSA discriminatory WB	ALRQ/ALRQ	Atypical scrapie, no signs of BSE
5	2008	Fallen stock	sheep	?	IDEXX Herdcheck, positive	Not done due to autolysis.	Positive for PrP by IDEXX Herdcheck. Atypical scrapie by AFSSA discriminatory WB	ARR/AHQ	Atypical scrapie, no signs of BSE
6	2010	Fallen stock	sheep	8	IDEXX HerdCheck, positiv	Not done due to autolysis.	Positive for PrP by IDEXX HerdCheck. Atypical scrapie by AFSSA discriminatory WB	AHQ/ARQ	Atypical scrapie, no signs of BSE
7	2010	Fallen stock	sheep	10	IDEXX HerdCheck, positive	Not done due to autolysis.	Positive for PrP by IDEXX HerdCheck. Atypical scrapie by AFSSA discriminatory WB	Not possible due to autolysis	Atypical scrapie, no signs of BSE
8	2011	Fallen stock	Sheep	15	IDEXX HerdCheck, positive	PrP staining Cerebellum <<< obex	Positive for PrP by IDEXX HerdCheck. Atypical scrapie by AFSSA discriminatory WB	AHQ/AHQ	Atypical scrapie, no signs of BSE
9	2011	Fallen stock	Sheep	5	IDEXX HerdCheck, positive	Not done due to autolysis.	Positive for PrP by IDEXX HerdCheck. Atypical scrapie by AFSSA discriminatory WB	AFRQ/ARFQ	Atypical scrapie, no signs of BSE
10	2011	Fallen stock	sheep	13	IDEXX HerdCheck, positive	Not done due to autolysis.	Positive for PrP by IDEXX HerdCheck. Atypical scrapie by AFSSA discriminatory WB	AHQ/ARQ	Atypical scrapie, no signs of BSE
11	2011	Fallen stock	sheep	9	IDEXX HerdCheck, positive	Not done due to autolysis.	Positive for PrP by IDEXX HerdCheck. Atypical scrapie by AFSSA discriminatory WB	Not possible due to autolysis	Atypical scrapie, no signs of BSE
12	2011	Fallen stock	sheep	8	IDEXX HerdCheck, positive	PrP staining Cerebellum <<< obex	Positive for PrP by IDEXX HerdCheck. Atypical scrapie by AFSSA discriminatory WB	AHQ/AHQ	Atypical scrapie, no signs of BSE
13	2016	Fallen stock	sheep	16	IDEXX HerdCheck, positive	Not done due to autolysis.	APHA Biorad Hybrid Western blot***	Not possible due to autolysis	Atypical scrapie, no signs of BSE

* Analyses done at the EU Community Reference laboratory for TSE, VLA, Weybridge, UK: The molecular profiles for both cases exhibited an atypical profile compared to the classical scrapie positive control. Conclusion: Atypical scrapie, no signs of BSE.
**VET.DTU has so far employed the VLA Hybrid Western Blotting technique as the discriminatory test to exclude BSE from TSE cases of small ruminants. The technique is, however, not suitable for atypical scrapie cases, due to the harsh protease digestion conditions, and VET-DTU is presently implementing an EU-approved, AFSSA Western Blotting technique, which will provide a signal from atypical scrapie. Preliminary experiments using mild PK treatment on the isolate 2006-10-2826 demonstrated a strong response from the antibody P4 in Western Blotting with a band pattern covering a broad MW-range, indicating that the TSE in question was not BSE. The concluding diagnosis: Atypical scrapie, was established according to the guidelines in the EFSA Opinion of October 26, 2005, by the following set of observations:
1) A positive response in the IDEXX HerdCheck test from both brain stem and cerebellum
2) A massive immunohistochemistry reaction in sections of cerebellum and a weak response in sections of brain stem
3) A negative response in Prionics Western Blotting from both brain stem and cerebellum
The old age (7 years) and the genotype (AHQ/AHQ) of the animal supported the diagnosis further.
***) All material analysed at the EU Community Reference laboratory for TSE, APHA, Weybridge, UK,

Obex from a sheep with atypical scrapie. Detection of PrP by IHC. A fine granular, low grade, accumulation of PrP (brown) was observed.



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